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DETERMINATION OF NICOTINE AND ITS METABOLITES IN URINE BY HPLC AFTER DETBA DERIVATIZATION

Georg Schepers, Ph.D.*, Demetrios Demetriou, M.Sc., INBIFO Institut fuer biologische Forschung GmbH

A method for the simultaneous determination of nicotine and 9 of its metabolites, i.e., cotinine, 3'-hydroxycotinine, 5'-hydroxycotinine, nicotine-N'-oxide, norcotinine, nornicotine, 4-(3-pyridyl)-4-hydroxybutyric acid, 4-(3-pyridyl)-4-oxybutyric acid, and 3-pyridylacetic acid, in human urine is described. The phase-2 metabolites nicotine-N-glucuronide, cotinine-N-glucuronide, and 3'-hydroxy-O-glucuronide are determined in a 2nd chromatographic run after treatment of the urine sample with ß-glucuronidase and calculation of the difference between the free and total analytes. Approximately 97% of the known metabolites excreted in human urine can be determined.

The method requires no clean-up of the urine samples, e.g., by extraction, and is based on our method for HPLC analysis after DETBA derivatization [Rustemeier et al., *J. Chromatogr.* 613, 95-103, (1993)]. Modification of derivatization conditions, solvent composition, pH, flow, and detection wavelengths has resulted in significant improvements in the method. Derivatization and chromatography are fully automated and the chromatography is performed within 13.5 min.

The method was used for biomonitoring of the nicotine uptake and the determining of the relative distribution pattern of nicotine and its metabolites in urine of smokers.

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Georg Schepers, INBIFO Institut fuer biologische Forschung GmbH, Fuggerstr. 3, D-51149 Cologne, Germany; e-mail: schepers.georg@pmintl.ch